Brain Responses to Disorder-Related Visual Threat in Panic Disorder

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Abstract: Panic disorder (PD) patients show aberrant neural responses to threatening stimuli in an extended fear network, but results are only partially comparable, and studies implementing disorder-related visual scenes are lacking as stimuli. The neural responses and functional connectivity to a newly developed set of disorder-related, ecologically valid scenes as compared with matched neutral visual scenes, using event-related functional magnetic resonance imaging (fMRI) in 26 PD patients and 26 healthy controls (HC) were investigated. PD patients versus HC showed hyperactivation in an extended fear network comprising brainstem, insula, thalamus, anterior, and mid-cingulate cortex and (dorso-)medial prefrontal cortex for disorder-related versus neutral scenes. Amygdala differences between groups failed significance. Subjective levels of anxiety significantly correlated with brainstem activation in PD patients. Analysis of functional connectivity by means of beta series correlation revealed no emotion-specific alterations in connectivity in PD patients versus HC. The results suggest that subjective anxiety evoked by external stimuli is directly related to altered activation in the homeostatic alarm system in PD. With novel disorder-related stimuli, the study sheds new light on the neural underpinnings of pathological threat processing in PD. *Hum Brain Mapp* 37:4439–4453, 2016. © 2016 Wiley Periodicals, Inc.

Key words: panic disorder; disorder-related scenes; fMRI; brainstem

Additional Supporting Information may be found in the online version of this article.

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INTRODUCTION

Recurring sudden panic attacks and anxious apprehension are the two core symptoms for diagnosing panic disorder [American Psychiatric Association, 2000]. A panic attack is characterized by extreme bodily symptoms such as sensation of racing or pounding heartbeat, chest pains, nausea, dizziness, trembling, or shaking, often accompanied by the urge to escape or the fear of dying. Lifetime prevalence for PD (with and without agoraphobia) is 4.7% [Kessler et al., 2006]. PD patients report substantial impairment in quality of life [Barrera and Norton, 2009], highlighting the need to better understand this disorder.

To gain a neurobiological understanding of PD, Gorman et al. [2000] revised their earlier hypothesis [Gorman et al., 1989] that brainstem, prefrontal cortex (PFC), and limbic structures represented different characteristics of the disorder, proposing an amygdala-centered fear network in PD instead. Recent functional magnetic resonance imaging (fMRI) studies on affective processing in PD point to an even broader fear network, including amygdala, brainstem, hypothalamus, thalamus, insula, anterior cingulate cortex (ACC), and PFC [de Carvalho et al., 2010; Dresler et al., 2013; Duval et al., 2015; Shin and Liberzon, 2009]. Remarkably, amygdala activation is reported only infrequently [see also Etkin and Wager, 2007].

Neurological and recent neurobiological findings support this broader network, and parallel pathways in PD [Geiger et al., 2014; Pannekoek et al., 2013]. Altered activation in these regions in PD patients has underlined the regions' role in the pathophysiology of the disorder. In PD, amygdalar dysfunction may contribute to development and progression of the disorder, as it is related to increased conditionability, resistance to extinction, irrational fear of attacks, and avoidance behavior [for a review see Kim et al., 2012]. The brainstem, central to homeostatic regulation such as cardiorespiratory functions, is a pivotal but scarcely studied structure in PD [Perna et al., 2014]. In an emotional interference task, Chechko et al. [2009] found increased brainstem activation in PD patients versus HC. Comparing PD with PTSD patients and HC, Tuescher et al. [2011] reported decreased brainstem activation in the threat condition of an instructed fear paradigm, while increased brainstem activation was found in the safe condition. Overall, relative to HC, PD patients seem to show abnormal brainstem activation during emotional processing, underlining the role of the brainstem in PD pathophysiology. In healthy subjects, brainstem structures play a role in subjective anxiety and immediate defensive reactions, such as the urge to escape [Mobbs et al., 2007, 2009]. The thalamus, an important relay between peripheral and cortical sensory signals, responds in a hypersensitive way to threatening stimuli in anxiety-disorder patients [for a review see Duval et al., 2015]. Next, the insula is involved in processing of interoceptive information [Critchley et al., 2004; Paulus and Stein, 2006], emotional behavior in general, and in the generation of subjective feelings of emotion [for a review see Gasquoin, 2014]. ACC and medial PFC (mPFC) are both involved in aspects of negative-emotion processing, such as appraisal, expression, and regulation of emotion [Etkin et al., 2011]. Hyperactivation in (dorsal) ACC has been reported across anxiety disorders, and interestingly, is also observed in fear conditioning [for a review see Shin and Liberzon, 2009], a mechanism that plays a pivotal role in the etiology of PD [Bouton et al., 2001; Grillon et al., 2007; Lissek et al., 2005, 2009]. Moreover, in their review of neurobiological findings in PD, Graeff and Del-Ben [2008] propose ACC and insula to be involved in the detection of interoceptive cues.

Next to area-related brain activation measures, studies investigating task-related connectivity in PD are emerging. Connectivity analyses between the above mentioned regions, which comprise the extended and revised fear network in PD, are crucial to gain a better understanding of neural correlates of PD pathophysiology. Comparing amygdala–mPFC connectivity in PD and social phobia patients during face processing by means of psychophysiological interaction analysis, Demenescu et al. [2013] could not reveal any effect of diagnosis. Task-related connectivity was further assessed in a randomized controlled trial studying the role of cognitive-behavioral therapy (CBT) on connectivity in aversive conditioning [Kircher et al., 2013; Lueken et al., 2013]. Increased positive inferior frontal gyrus (IFG) coupling with amygdala, insula, and ACC was found before and after psychotherapy [Kircher et al., 2013]. This finding was confirmed by Lueken et al. [2013] who additionally found a negative coupling of ACC and amygdala in CBT-responders, while non-responders showed positive ACC-amygdala-coupling. These two studies suggested stable fronto-limbic connectivity to be either a trait factor in, or risk factor for, PD. In line with this idea of altered connectivity as risk factor, insula-amygdala coupling has been reported in subjects with a premorbid risk factor for psychiatric disorders [Klucken et al., 2015].

Taken together, no fully coherent picture emerges from neuroimaging findings in PD. Besides differences in experimental designs and analytical methods, studies differ vastly with regard to stimulus material. Fearful faces [Demenescu et al., 2013; Domschke et al., 2006; Ottaviani et al., 2012; Pillay et al., 2006], general threat-related words [Maddock et al., 2003], or panic-related words [Dresler et al., 2012; Van den Heuvel et al., 2005] have been used to study threat-related processing in PD. More than words, visual information seems to trigger panic reactions. From a neuroscientific and clinical perspective, the crucial question is thus whether disorder-related visual cues with high ecological validity trigger the fear network previously identified in fMRI studies on PD. A first attempt to disorder-related visual processing in PD patients was made by Pauli et al. [1996], who developed a picture set of three emergency situations that were repeatedly used in behavioral [Pauli et al., 1996, 2001] and psychophysiological studies [Amrhein et al., 2005; Wiedemann et al., 1999]. Two other studies investigated agoraphobic scene processing in patients suffering from PD with agoraphobia. With a standardized set of agoraphobic scenes, Wittmann et al. [2014] found hyperactivation of ventral striatum and insula when PD patients with agoraphobia anticipated agoraphobia-related situations.

A set of ecologically valid stimuli for PD (with or without agoraphobia) is still lacking. We developed such a standardized set of complex disorder-related visual scenes, complemented by neutral scenes of comparable complexity. We investigated neural correlates of disorder-related visual scene processing, comparing PD patients and HC.

TABLE I. Demographic and clinical characterization

	PD $M \pm SD$	HC $M \pm SD$	t -value/ χ^2 -value	P -value
N (female/male)	20/6	19/7	$\chi^2 = 19.33$	0.252
Age, years	24.88 ± 6.12 (18–46)	23.96 ± 3.18 (19–32)	$t = -0.682$	0.499
Years of education	12.5 ± 0.99	12.44 ± 1	$t = -0.215$	0.831
MWT-B	27.50 ± 11.58	28.08 ± 3.29	$t = 0.244$	0.190
ethnicity	Caucasian	Caucasian		
Employment status (employed/self- employed/student/seek- ing work/retired)	4/0/22/0/0	4/0/22/0/0	$\chi^2 = 0.00$	1.00
DSM-IV diagnosis				
PD (DSM 300.01)	12	N/A		
PD with A (DSM 300.21)	14	N/A		
Therapeutic status (no therapy/therapy)	22/4	N/A		
Psychiatric medication	None	None		
Questionnaire data				
PAS	20.81 ± 6.97	N/A		
ACQ	$2.17 \pm .59$ (1.07–3.21)	1.22 ± 0.16 (1–1.60)	$t = -7.922$	<0.001
BSQ	2.88 ± 0.69 (1.76–4.65)	1.44 ± 0.32 (1–2.22)	$t = -9.683$	<0.001
MI _{total}	1.81 ± 0.63 (1–3.41)	1.13 ± 0.16 (1–1.57)	$t = -5.278$	<0.001
BDI	13.92 ± 8.73 (0–36)	0.58 ± 1.27 (0–6)	$t = -7.717$	<0.001
Comorbidities				
Mild depressive episode (DSM 296.21)	4			
Generalized anxiety dis- order (DSM 300.02)	2			
Somatization disorder and hypochondria (DSM 300.81; DSM 300.7)	2			
Social or specific phobia (DSM 300.23; DSM 300.29)	3			
Bulimia nervosa (DSM 307.51)	1			
Obsessive-compulsive disorder (DSM 300.3)	1			

Note. PD, Panic Disorder patients; HC, healthy controls; M = Mean; SD = standard deviation. MWT-B, Multiple-Choice Vocabulary Intelligence Test, Version B; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; PAS, Panic and Agoraphobia Scale; ACQ, Agoraphobic Cognitions Questionnaire; BSQ, Body Sensations Questionnaire; MI, Mobility Inventory for Agoraphobia; BDI, Beck Depression Inventory.

Regions of interest (ROIs) were based on models of emotional stimulus processing in PD [de Carvalho et al., 2010; Dresler et al., 2013; Gorman et al., 2000]: brainstem, amygdala, insula, thalamus, anterior, and mid-cingulate cortex (ACC and MCC) and medial prefrontal cortex (PFC). Disorder-related scenes were expected to be rated as more unpleasant, more arousing and more anxiety-inducing than neutral scenes in PD patients than in HC. Furthermore, we hypothesized hyperactivation to disorder-related versus neutral scenes (disorder-related > neutral) in the mentioned ROIs in PD patients as compared with HC. We additionally investigated correlations between the effects and individual anxiety ratings for the scenes. To further clarify the network underlying disorder-related scene

processing in PD, functional connectivity was assessed by means of beta series correlations (BSC). Based on the literature, amygdala, insula, and ACC served as seed regions for BSC [Kircher et al., 2013; Lueken et al., 2013, 2015].

MATERIALS AND METHODS

Subjects

PD patients were recruited at the psychotherapeutic outpatient clinic of the University of Muenster and by public announcements. HC participants were drawn from a larger number of screened healthy controls ascertained within the framework of the Collaborative Research Center “Fear,

Anxiety, Anxiety Disorders" (TRR SFB 58; <http://sfbtrr58.uni-muenster.de/>) or recruited by means of flyers and newspaper ads. Inclusion criteria for all participants were German as native language, normal or corrected-to-normal vision, and right-handed as assessed with the Edinburgh Handedness Inventory [Oldfield, 1971]. Exclusion criteria for both groups were psychiatric medication, neurological disorders, presence or history of psychotic or bipolar disorder, drug dependence or abuse within the last 10 years, suicidal ideations, and fMRI contraindications. From the 27 PD patients who took part in the present study, one patient's data had to be discarded due to technical problems in button-press recording. The final sample comprised 26 PD patients (age range: 18–46 years) and 26 HC (age range: 19–32 years), matched for age, gender and education. Twelve of the twenty-six PD patients had a primary diagnosis of PD [DSM 300.01; American Psychiatric Association, 2000], fourteen patients had a primary diagnosis of PD with agoraphobia (DSM 300.21). Four of the twenty-six PD patients underwent psychotherapy at the time of the study. The Panic and Agoraphobia Scale (PAS) scores of these four patients indicated mild to moderately severe impairment [Bandelow, 1997]. Controls were free of any psychiatric diagnosis. Sample characteristics are provided in Table I. All subjects gave written informed consent. The study conforms to the Declaration of Helsinki and was approved by the ethics committee of the University of Muenster.

Overall Procedure

The study comprised three sessions, completed on separate days: psychopathological assessment, fMRI experiment, and post-scanning stimulus rating.

Psychopathological assessment

Prior to participation, all patients and controls were interviewed by an experienced clinical psychologist, using the Structured Clinical Interview for DSM-IV Axis I Disorders [SCID; Wittchen et al., 1997]. PD symptom severity, trait anxiety and levels of depressive mood were assessed with self-rating questionnaires [Panic and Agoraphobia Scale (PAS); Bandelow, 1997]; Agoraphobic Cognitions Questionnaire, Body Sensations Questionnaire and Mobility Inventory [ACQ; BSQ; MI; Ehlers et al., 2001], Beck Depression Inventory [BDI; Hautzinger et al., 1995] (see Table I). Subjects received standardized instructions and a 5-min task training outside the scanner.

fMRI experiment

A 1-min training was conducted inside the scanner. During the 8 min 19 s functional run, each of the 50 disorder-related and 50 neutral pictures was presented once in an event-related design (see below for stimulus details). Pictures of neutral and disorder-related scenes

were presented in random sequence, optimized and counterbalanced with the Optseq algorithm (<https://surfer.nmr.mgh.harvard.edu/optseq/>), which implements temporal jitter to enhance signal discriminability by increasing variability in the hemodynamic response function [Burock et al., 1998; Dale et al., 1999]. Four different randomizations of the order of stimuli were implemented to counteract possible sequence effects. Stimuli were presented for 800 ms, separated by a temporal gap with an average duration of 3,915 ms (jittered between 1,280 and 15,320 ms) during which a white fixation cross was presented. To direct and keep participants' attention to the presented scenes, they performed a vigilance task which required pressing a button with their right index finger when a blurred scene occurred (five trials). Five blurred scenes (originally EmoPicS, [Wessa et al., 2010] blurred with Adobe Photoshop CS6 [version 13.0.1, Adobe Systems Inc., San Jose, CA]) were randomly presented over the course of the experiment.

Post-scanning stimulus rating

Participants rated all 100 scenes within 7 days after the fMRI experiment on a computer outside the scanner. A nine-point Likert scale was used to assess valence (1 = very unpleasant, 5 = neutral to 9 = very pleasant), arousal (1 = not arousing to 9 = very arousing), and anxiety (1 = not anxiety inducing to 9 = very anxiety inducing). After 2 s stimulus presentation, participants rated the scenes with regard to valence, arousal and anxiety. Rating data were analyzed by means of 2×2 repeated-measures analyses of variance (ANOVAs) with *Scene Type* (disorder-related, neutral) as a within-subject factor and *Group* (PD, HC) as a between-subject factor. Statistical significance was set to $P < 0.05$, and post-hoc comparisons were Bonferroni-corrected for multiple testing.

Stimuli

Pilot-study development of standardized stimulus set, the Panic-related Picture Set Muenster (PAPS-M)

The stimulus set used in the present study was based on a pilot study, consisting of a web search followed by an expert and a patient evaluation of the visual scenes. First, pictures of disorder-related scenes were collected in an extensive web search, using key words related to bodily symptoms characteristic of PD (e.g., shortness of breath, hyperventilation, heart palpitations, chest pain, trembling or shaking, feeling dizzy, fainting), panic-related fears (tumor, heart attack), and agoraphobia-related place descriptions (crowded bus, dark tunnel, glass elevator). The search yielded 171 pictures. Second, 10 clinical experts were asked to categorize the 171 pictures with regard to their suitability to elicit anxiety in a panic disorder patient (response options: "unsuitable," "rather unsuitable," "rather suitable," "suitable"). Inter-rater reliability among

the 10 clinical experts was found to be strong (Intra-Class-Correlation (3,10) = 0.821, $P < 0.001$). Third, the 96 scenes rated highest by the clinical experts were evaluated by seven HC (2 males, mean age = 34.43; SD = 13.29; mean PAS score = 0, SD = 0) and seven PD patients (1 male, mean age = 31.43; SD = 13.28; mean PAS score = 17.29, SD = 9.76), who did not participate in the main study. For each of these 96 pictures the differential anxiety rating between PD patients and HC was calculated. The 50 scenes which discriminated best between PD patients and HC were selected as the final set. Fifty neutral scenes were matched with the fifty final panic scenes. Neutral scenes were taken from the IAPS [Lang et al., 2008] and EmoPics databases [Wessa et al., 2010]. Five additional scenes (all selected from EmoPics), manipulated (“blurred”) by means of Adobe Photoshop, were used in the decision task (see above). Neutral and disorder-related scenes did not differ (all $P > 0.05$) with respect to content-related (e.g., indoor/outdoor) or physical criteria (e.g., red-green-blue value, brightness, product entropy) apart from the panic-related content (see Supporting Information Table S1, PAPS-M is available for use by the scientific community).

FMRI Acquisition and Analysis

BOLD (blood oxygenation-level-dependent) responses and structural brain information were recorded using a 3 Tesla magnetic resonance scanner (“Magnetom PRISMA,” Siemens, Medical Solutions, Erlangen Solutions, Erlangen, Germany) and a 20-channel Siemens Head Matrix Coil. First, a localizer was conducted followed by a high resolution T1-weighted anatomical scan. Magnetization Prepared Rapid Gradient Echo (MP-RAGE) data (192 axial slices of 1 mm thickness, gap = 0 mm, in plane resolution = $1 \times 1 \text{ mm}^2$) were acquired with a repetition time (TR) of 2,130 ms, an echo time (TE) of 2.28 ms, a flip angle of 8° , and an acquisition matrix of $92 \times 92 \text{ mm}$. Images were obtained within a field of view (FOV) of 256 mm. A functional run of 255 volumes was conducted using a T2*-weighted echo-planar sequence (TE = 30 ms, flip angle = 90° , matrix = $92 \times 92 \text{ voxels}$, FOV = 208 mm, TR = 2,080 ms). Each volume consisted of 36 axial slices (thickness = 3 mm, gap = 0.3 mm, in plane resolution = $2.26 \times 2.26 \text{ mm}^2$). The functional images were scanned using an ascending interleaved sequence. To minimize susceptibility artifacts in inferior parts of anterior brain areas, the volumes were tilted approximately 20° from the AC/PC line. A shim-ming field was applied before functional imaging to minimize external magnetic field inhomogeneities.

FMRI data were analyzed with BrainVoyager QX software (Version 2.4; Brain Innovation, Maastricht, The Netherlands). The first ten volumes of each run were discarded from analysis to ensure steady-state tissue magnetization. First, all volumes were realigned to the first volume in order to minimize artifacts due to head movements. Volumes were then resampled to a voxel size of $2 \times 2 \times$

2 mm, and slice-time correction was applied. Further data preprocessing steps comprised spatial (6 mm full-width half-maximum isotropic Gaussian kernel) as well as temporal smoothing (high pass filter: 10 cycles in time course; low pass filter: 2.8 s; linear trend removal). The anatomical and functional images were co-registered and normalized to Talairach space [Talairach and Tournoux, 1988]. Normalization procedure was used as implemented by default in BrainVoyager.

For statistical analyses, multiple linear regressions modeling the signal time course at each voxel were calculated. The expected BOLD signal change for each predictor was modeled with a canonical double gamma hemodynamic response function (HRF). Predictors of interest were disorder-related and neutral scenes. The five trials with blurred scenes as well as the six motion parameters were included as regressors into the model. The latter were included to account for movement artifacts of the participants. Neither root mean squared values for the six translation and rotation axes nor the single summary statistic root mean squared head position differed significantly between the two groups (all $P > 0.1$). Statistical comparisons were conducted using a mixed-effect analysis. First, voxel-wise statistical maps were generated and percent-standardized predictor estimates (beta weights) were computed for each subject. Predictor estimates were analyzed across subjects by means of t-tests in specific regions of interest (ROI).

Brainstem, amygdala, insula, thalamus, ACC, MCC, and mPFC served as ROIs. Masks of all ROIs were combined into a single mask and cluster threshold estimation was carried out across this one mask (small volume correction). The Automated Anatomical Labeling (AAL) atlas included in the Wake Forest University pick atlas (WFU) [Maldjian et al., 2003; Tzourio-Mazoyer et al., 2002] provided local information for ROIs [amygdala (dilated 1 mm in radius), insula (dilated 1 mm in radius), thalamus, medial prefrontal cortex, ACC, and MCC]. For the mPFC-ROI the two AAL templates “Frontal_Med_Orb” and “Frontal_Sup_Medial” were used. ROIs for the brainstem were downloaded from the digitized version of the Talairach atlas (<http://www.talairach.org/nii/gzip/>). For all regions, the obtained MNI coordinates were converted to Talairach space in Matlab (version 8.2, The MathWorks Inc., Natick, MA) using the ICBM-152 routine proposed by Lancaster et al. [2007]. Labeling of peak voxel was verified by the Mai atlas [Mai et al., 2004] and supported by the Talairach Dameron Software [Lancaster et al., 2000; <http://www.talairach.org/client.html>]. Statistical parametric maps as derived from the voxel-wise analysis were considered significant for clusters surviving cluster-based correction for multiple comparisons. Voxel-level threshold was initially set to $P < 0.005$ (uncorrected) to balance between type I and type II error type [Lieberman and Cunningham, 2009]. All statistical analyses were performed using cluster-based Monte Carlo simulations (1,000 iterations,

Figure 1.

Mean post-scanning ratings for valence, arousal and anxiety according to disorder-related and neutral scenes for panic disorder patients (PD) and healthy controls (HC). Ratings were given

on 9-point Likert scales as follows: valence, 1 = negative, 5 = neutral, 9 = positive; arousal, 1 = calm, 9 = intense; anxiety, 1 = low, 9 = high. * $P < 0.025$.

cluster-level $\alpha = 0.05$) to control for multiple testing [Goebel et al., 2006]. Labeling of peak voxel was supported by the Talairach Dameon Software [Lancaster et al., 2000; <http://www.talairach.org/client.html>].

Correlation analysis

Among PD patients, correlations between differential anxiety ratings for disorder-related versus neutral scenes and extracted mean beta values within the clusters showing differential neural effects for *Scene Type* were calculated. As there were ten comparisons, Bonferroni correction for multiple comparisons was applied ($P < 0.005$).

Beta series correlation (BSC)

As a measure of condition-related functional connectivity, the beta series correlation method [Rissman et al., 2004] was applied using single trial parameter estimates obtained by least squares estimation [Mumford et al., 2012]. Analyses of condition-related functional connectivity were conducted in Matlab (version 8.2, The MathWorks Inc., Natick, MA). Each individual trial of interest was modeled as a separate regressor in a General Linear Model (GLM) with all other disorder-related and neutral trials ($n = 99$), the five blurred scene trials and the six motion parameters serving as nuisance regressors. Since the current experiment comprised 50 disorder-related and 50 neutral trials, we obtained a series of 50 beta values for each voxel, condition, and participant. For the next step, amygdala, insula, and ACC were used as seed regions, which were created by masks of the WFU pick atlas as described above [amygdala (dilated 1 mm in radius), insula (dilated

1 mm in radius), ACC; Maldjian et al., 2003]. For each participant and condition, the series of beta values from a given seed region was averaged across the seed region and regressed on the series of beta values in each of the remaining voxels (apart from voxels in the seed region and its closest neighborhood, defined by an isomorph inflation of the seed region by approximately 3 voxels). This resulted in correlation maps for each condition for each participant between a seed region and all other voxels. To allow for statistical comparison, the resulting correlation coefficients were Fisher's z transformed [Fisher, 1915]. Cluster permutation was conducted on these connectivity z maps. We conducted 2×2 analyses of variance (ANOVAs) with *Scene Type* (disorder-related, neutral) as a repeated measurement factor and *Group* (PD, HC) as a between-subject factor on these connectivity z maps. Within each group, the connectivity difference z maps of patients and HC were compared with a paired sample t -test. As there were six seed regions, Bonferroni correction for multiple comparisons was applied ($P < 0.008$, six comparisons).

RESULTS

Rating Data: Valence, Arousal, and Anxiety Ratings

Mean rating of valence, arousal, and anxiety for *Scene Type* (disorder-related, neutral) and *Group* (PD, HC) are provided in Figure 1 (see Supporting Information Table S2 for details). Valence ratings showed a significant main effect of *Group* ($F(1,50) = 6.79$, $P = 0.012$), with higher unpleasantness values for PD than for HC. A significant main effect of *Scene Type* ($F(1,50) = 255.49$, $P < 0.001$)

TABLE II. Significant hyperactivation for disorder-related as compared with neutral stimuli in panic disorder patients (PD) versus healthy controls (HC) for a priori defined regions of interest

Region	Lateralization	Talairach coordinates of peak voxel			MNI coordinates of peak voxel			Cluster size (mm ³)	<i>t</i> -value average	<i>t</i> -value maximum
		<i>x</i>	<i>y</i>	<i>z</i>	<i>x</i>	<i>y</i>	<i>z</i>			
PD> HC, disorder-related > neutral										
Brainstem	L	−1	−32	−23	−3	−31	−32	144	3.06	3.52
Thalamus	L	−20	−25	−3	−21	−24	−9	656	3.22	4.47
Thalamus	R	16	−24	2	17	−23	−2	184	3.05	3.68
Insula	L	−34	8	−6	−35	12	−11	936	3.10	4.16
Insula	R	36	0	2	38	2	−2	336	3.14	4.00
MCC	L	−8	−2	33	−9	−4	34	296	3.25	3.97
posterior MCC	R	10	−17	37	10	−19	40	176	3.00	3.31
MPFC	R	9	24	54	9	20	60	336	3.08	3.83
MPFC	R	10	50	32	11	51	36	384	3.02	3.54
dmPFC/ACC	L	−5	46	13	−5	50	15	344	2.97	3.47
HC> PD, disorder-related > neutral										
None										

Note. IFG inferior frontal gyrus, MPFC middle prefrontal cortex, SFG superior frontal gyrus. Regions listed were thresholded at $P < 0.005$ (uncorrected), $P < 0.05$ (corrected). L = left, R = right.

showed that disorder-related scenes were rated as more unpleasant than neutral scenes. The *Group × Scene Type* interaction failed significance ($F(1,50) = 2.83$; $P = 0.099$).

Analysis of arousal ratings revealed significant main effects for *Group* ($F(1,50) = 6.49$, $P = 0.014$) and *Scene Type* ($F(1,50) = 227.34$, $P < 0.001$). PD patients rated stimuli as more arousing than HC, and disorder-related scenes were rated as more arousing than neutral scenes. The *Group × Scene Type* interaction reached marginal significance ($F(1,50) = 3.66$; $P = 0.061$). Applying the Bonferroni-corrected significance level for multiple comparisons ($P < 0.025$), analyses revealed levels of arousal to be higher in PD patients than in HC, but only for disorder-related ($t(50) = 2.55$, $P = 0.014$), not for neutral scenes ($t(50) = 2.09$, $P = 0.042$).

For anxiety ratings, there was a significant main effect of *Group* ($F(1,50) = 8.8$, $P = 0.005$), with PD patients reporting higher anxiety levels than HC. A significant main effect of *Scene Type* ($F(1,50) = 127.15$, $P < 0.001$) showed that disorder-related scenes were associated with higher anxiety levels than neutral scenes. Importantly, the *Group × Scene Type* interaction was also significant ($F(1,50) = 10.05$; $P = 0.003$), with significantly larger anxiety levels in PD patients than in HC specifically for disorder-related, but not for neutral scenes (PDdisorder-related > HCdisorder-related; $t(50) = 3.12$, $P = 0.003$, PDneutral vs. HCneutral, $t(50) = 1.56$, $P = 0.125$).

FMRI Data

Compared with HC, PD patients displayed greater activation to disorder-related vs. neutral scenes in brainstem, bilateral insula, bilateral thalamus and bilateral MCC. A cluster reaching into ACC and dorsomedial PFC as well as

two clusters in the right medial PFC showed greater activation for PD patients versus HC in the contrast disorder-related versus neutral scenes (see Table II and Fig. 2, for further details on analysis separately for each group see Supporting Information Table S3 and S4). Further inspection of amygdala activation revealed a hyperactivation in right amygdala to disorder-related versus neutral scenes for PD patients versus HC that failed significance (right amygdala, $x = 23$, $y = -2$, $z = -18$, cluster size: 65 mm³, maximal *t*-value: 2.97, $P < 0.005$ uncorrected).

Correlation analysis

A highly significant correlation of activation in the brainstem in PD patients and differential anxiety ratings (disorder-related minus neutral scene ratings) was revealed ($r = 0.598$, $P = 0.001$, $R^2 = 0.358$, Bonferroni corrected; see Fig. 3).

Whole brain analysis

Effects in bilateral insula, bilateral thalamus, bilateral MCC, left ACC/dmPFC, and right mPFC were also present in a whole brain analysis. Additional clusters revealed by whole brain analysis were located in dorsolateral PFC, frontal gyrus, precentral gyrus, posterior cingulate cortex (PCC), precuneus, inferior parietal lobe, and temporal gyrus (Table III, for further details on analysis separately for each group see Supporting Information Table S5 and S6). HC showed greater activation than PD patients to disorder-related versus neutral scenes in the caudate.

Figure 2.

Differential brain activation for disorder-related compared with neutral stimuli in panic disorder patients (PD) versus healthy controls (HC) in a priori defined ROIs (PD > HC, disorder-related > neutral, all $P < 0.005$ uncorrected; $P < 0.05$ corrected). Black bars display parameter estimates for PD, white bars for HC. PD patients display

an enhanced activation in brainstem, bilateral thalamus, bilateral insula, bilateral midcingulate cortex (MCC), right medial prefrontal cortex (MPFC), and dorsomedial prefrontal cortex/anterior cingulate cortex (ACC). All figures displayed at $P < 0.005$. [Color figure can be viewed at wileyonlinelibrary.com]

Beta series correlation (BSC)

To examine condition-related functional connectivity in PD patients versus HC, we compared BSC for disorder-related versus neutral trials, represented in connectivity difference z maps. We found no significant emotion-specific differences between PD patients and HC in functional connectivity. No effect of *Scene Type* was revealed within each group.

DISCUSSION

This study investigated neural correlates of disorder-related visual scene processing in PD patients with a large new disorder-related visual stimulus set that elicited anxiety in PD patients but not HC. Results show

Figure 3.

Correlation between brainstem beta values in panic disorder patients in the contrast *Scene Type* and individual anxiety ratings (disorder-related minus neutral). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE III. Significant hyperactivation for disorder-related as compared with neutral stimuli in PD versus HC in as found in whole brain analysis

Region	Lateralization	Talairach coordinates of peak voxel			MNI coordinates of peak voxel			Cluster size (mm ³)	<i>t</i> -value average	<i>t</i> -value maximum
		<i>x</i>	<i>y</i>	<i>z</i>	<i>x</i>	<i>y</i>	<i>z</i>			
PD > HC, disorder-related > neutral										
Thalamus	L	−19	−25	−3	−20	−24	−9	888	3.19	4.47
Thalamus	R	16	−24	2	17	−23	−2	184	3.05	3.68
Insula	L	−34	9	−7	−35	13	−12	944	3.09	4.16
Insula	R	37	0	2	39	2	−2	336	3.14	4.00
MCC	L	−7	−2	34	−8	−3	36	296	3.25	3.97
posterior MCC	R	11	−19	38	11	−21	41	280	2.97	3.31
MPFC	R	8	23	53	8	19	58	976	3.13	3.95
MPFC	R	10	50	32	11	51	36	672	2.94	3.54
dmPFC/ACC	L	−6	46	13	−6	49	15	344	2.97	3.47
dIPFC	L	−28	21	36	−29	20	39	1,472	3.08	4.16
dIPFC	R	31	12	45	31	9	48	1,688	3.22	5.13
SFG	R	16	2	61	16	−2	68	520	3.01	3.58
IFG	L	−34	29	1	−35	33	1	288	2.91	3.35
IFG	R	39	22	−16	41	27	−23	4,488	3.18	4.34
Precentral Gyrus	R	33	−24	33	33	−26	34	776	2.91	3.35
Precentral Gyrus	R	46	−11	20	48	−11	19	256	3.10	3.91
PCC	L	−1	−52	22	−3	−54	21	600	2.84	3.31
PCC	L	−2	−52	22	−3	−54	21	600	2.85	3.31
PCC	R	10	−56	11	10	−58	8	736	2.91	3.32
Inferior parietal lobe	L	−59	−43	24	−61	−44	23	624	3.02	3.58
Precuneus	L	−10	−46	46	−11	−49	50	224	2.85	3.10
Precuneus	R	6	−53	41	6	−56	45	280	2.92	3.24
STG	L	−28	9	−22	−28	14	−29	248	3.00	3.72
STG	R	48	10	2	51	13	−2	192	2.95	3.35
STG	R	63	−43	22	64	−45	21	208	2.89	3.29
STG	R	44	−21	−6	47	−19	−13	2,400	3.17	4.09
MTG	L	−42	−40	3	−45	−40	−1	232	3.11	3.71
MTG	L	−51	−12	−10	−54	−10	−16	392	2.83	3.25
MTG	R	38	7	−36	39	14	−45	728	3.10	3.95
MTG	R	52	3	−17	55	8	−25	768	3.06	3.81
ITG	R	52	−14	−29	55	−10	−38	376	3.08	3.89
HC > PD, disorder-related > neutral										
Caudate	L	−29	−43	10	−31	−44	7	528	−3.43	−5.07

Note. PCC posterior cingulate cortex, IFG inferior frontal gyrus, ITG inferior temporal gyrus, STG superior temporal gyrus, MFG middle frontal gyrus, MTG middle temporal gyrus, STG superior temporal gyrus, SFG superior frontal gyrus. Regions listed were thresholded at $P < 0.005$ (uncorrected), $P < 0.05$ (corrected). L = left, R = right.

hyperactivation to disorder-related versus neutral scenes in PD patients relative to HC in an extended network of emotion processing. We observed increased responses to disorder-related scenes in brainstem, insula, thalamus, ACC, MCC and (dorso-)medial PFC in PD. Brainstem activation in PD patients highly correlated with subjective levels of anxiety evoked by disorder-related scenes. Furthermore, analysis of connectivity patterns by means of beta series correlation revealed no emotion-specific alterations in connectivity in PD patients versus HC. The following discussion targets the role of each brain region implicated in threat processing in PD before integrative conclusions summarize the discussion.

Brainstem

We observed brainstem hyperactivation for disorder-related scenes in PD patients that highly correlated with the subjective level of anxiety evoked by disorder-related versus neutral scenes. The coordinates suggest spatial correspondence with the dorsal pons, more specifically the locus coeruleus [Keren et al., 2009], but due to limitations in fMRI resolution and the small size of pontine substructures, caution is advised. In addition to volumetric investigations reporting increased brainstem volume in PD patients [Fujiwara et al., 2011; Protopopescu et al., 2006; Uchida et al., 2008], affective processing studies reveal

enhanced midbrain and pons activation in a face-word interference task [Chechko et al., 2009 (remitted PD patients)], and elevated dorsal midbrain/mesal periaqueductal gray activation in the safe condition of a fear conditioning paradigm [Tuescher et al., 2011]. Our results go far beyond these findings, unveiling a link between altered activation in a region relevant in the regulation of cardio-respiratory functions, and subjective levels of anxiety evoked by an exteroceptively presented scene. The highly significant correlational finding indicates that disorder-related scenes trigger basic fear mechanisms, such as changes in cardio-respiratory functions, leading to the subjective experience of anxiety. This matches the concept of increased sensitivity to bodily symptoms, for example cardio-respiratory changes, in PD and might provide further evidence for the brainstem's role in the etiology of PD via the mediation of autonomic nervous system functions [Sinha et al., 2000].

Insula and Amygdala

Disorder-related scenes elicited increased insular activation in PD. In general, the insula has been related to interoceptive awareness, that is, the representation of one's own bodily symptoms [Craig, 2009; Critchley et al., 2004], which plays a major role in the maintenance and etiology of PD [for a review see Domschke and Dannlowski, 2010]. The insula rather contributes to the production of an affective state than to emotion regulation [Phillips et al., 2003]. In line with that, symptom-provocation studies in PD patients have repeatedly yielded insular hyperactivation [but see Boshuisen et al., 2002; for a review see Dresler et al., 2013]. An essential role for the insula in PD has been shown in animal models, neurochemical studies and human neuroimaging studies [Cameron et al., 2007; for a review see Graeff and Del-Ben, 2008]. The insular hyperactivation observed here implies that increased interoceptive processing of disorder-related scenes is even present when no explicit emotional judgement, self-referencing, or symptom provocation is required. Taken together, the present insular hyperactivation underlines the pivotal role of the insula in PD and alludes to the affective significance of the scenes.

Given the role of the amygdala proposed for the pathophysiology of PD, it seems puzzling that amygdalar hyperactivation only reached marginal significance. However, as Etkin and Wager [2007] stated, "in panic disorder, amygdalar hyperactivity appears to be the exception, rather than the rule" (p. 2). While fMRI studies on anticipatory anxiety [Boshuisen et al., 2002], emotional conflict [Chechko et al., 2009; Dresler et al., 2012] or spontaneous panic attacks [Pfleiderer et al., 2007] have revealed amygdalar hyperactivation in PD, hypoactivations were observed for fearful faces [Ottaviani et al., 2012; Pillay et al., 2006] and for angry, fearful, happy, and neutral faces [Demenescu et al., 2013]. It remains unclear whether

aberrant amygdala activation is simply not as characteristic of PD as proposed earlier, whether it is too weak to be detected with common thresholds, or whether specific methodological limitations, make it difficult to detect. Indeed, amygdalar hyperactivation seems to strongly depend on stimuli and experimental paradigms, sample heterogeneity and size, as well as on limitations of neuroimaging techniques [Holzschneider and Mulert, 2011; Kim et al., 2012; Shin and Liberzon, 2009]. Studies implementing an additional distracting task or using emotional stimuli as distractors seem to be more likely to detect amygdala activation [Dolcos and McCarthy, 2006; Straube et al., 2006]. Since there was no distraction task in the present study, participants might have had enough cognitive capacities to initiate processes related to emotion regulation [Silver et al., 2007].

Thalamus

The thalamus has generally been implicated in supplying arousal to facilitate awareness in emotion processing [Anders et al., 2004; Straube et al., 2010; Van der Werf et al., 2002]. Models of PD consider the thalamus to be a major relay station in viscerosensory information processing and interconnections of thalamus and brainstem are believed to be important in sensory processing [Gorman et al., 2000]. Increased thalamic activation in PD has been observed during anticipation of panic-related symptoms [Boshuisen et al., 2002]. Thus, the present thalamic hyperactivation in PD might reflect elevated transmission of incoming sensations, probably linked to increased levels of arousal triggered by disorder-related stimuli.

Prefrontal and Cingulate Cortex

Aside from anxiety-related salience effects and increased interoceptive processing, (dorso-)medial PFC and cingulate cortex hyperactivations hint at a more profound evaluation of disorder-related scenes in PD patients than in HC. Although the ACC's relevance for processing of negative emotions, especially in anxiety, is undeniable [Milad et al., 2007], the significance of ACC dysfunction in PD is yet unclear. Affective neuroimaging studies in PD reveal a heterogeneous picture, with hypoactivation during processing of fearful faces [Domschke et al., 2006; Pillay et al., 2006], and hyperactivation during processing of neutral and happy faces [Pillay et al., 2006, 2007], in anticipation of panic symptoms [Boshuisen et al., 2002], during imagery of anxiety-provoking situations [Bystritsky et al., 2001], and during disorder-related word processing [Van den Heuvel et al., 2005]. Considering the disorder-relatedness of stimuli used in these studies, we tentatively conclude that ACC hyperactivation in PD patients is stronger with more panic-specific stimulus properties or tasks leading to symptom provocation. The same might apply for posterior MCC findings, since posterior cingulate cortex

hyperactivation was also observed in disorder-related word processing in PD patients [Maddock et al., 2003]. Cingulate cortex hyperactivations can be linked to several aspects of emotional processing, with the ACC being involved in aversively motivated behavior, such as hyper-scanning and preparedness for action [Straube et al., 2007] as well as emotional awareness [Bush et al., 2000]. The MCC is further claimed to play an important role in the generation of emotion, especially in highly intense aversive states [Shackman et al., 2011] and the regulation of autonomic activity [Luu and Posner, 2003], a process suggested to be dysfunctional in PD.

It is commonly believed that the mPFC is involved in evaluative aspects of emotion [Ochsner and Gross, 2005]. Given our present findings, elevated (dorso-) medial PFC activation to disorder-related stimuli in PD patients might reflect appraisal and explicit threat evaluation [Etkin et al., 2011; Mechias et al., 2010]. In fear and anxiety, the mPFC is involved in attention to one's own emotional states and suppression of fear-related behavioral responses [Miller et al., 2005]. MPFC activation has also been linked to anticipation of interoceptive threat in subjects with high fear of somatic symptoms [Holtz et al., 2012]. Anticipation of interoceptive and exteroceptive threat have both been suggested to rely on a network of increased insula and dorsal ACC/dorsomedial PFC activation [Hamm et al., 2014; Lane et al., 1998]. These results correspond with the interpretation that in our sample increased interoceptive threat processing is induced by exteroceptive aversive events. Although no emotional judgement or regulatory process is explicitly required in our rapid event-related paradigm, voluntary as well as automatic emotion regulation could have occurred during scene presentation [Phillips et al., 2008]. Across anxiety disorders, hyperactivations in dorsomedial PFC have been suggested to indicate "an overcompensatory response in an effort to decrease excessive fear responding" [p. 120; Duval et al., 2015].

Whole-Brain Analysis

Effects in bilateral insula, bilateral thalamus, bilateral MCC, left ACC/dmPFC, and right mPFC were also present in a whole brain analysis. Moreover, whole-brain analysis revealed additional bilateral dorsolateral PFC, inferior and superior frontal gyrus, precentral gyrus, posterior cingulate cortex (PCC), precuneus, inferior parietal lobe, and superior and middle temporal gyrus hyperactivation in PD. These regions largely overlap with the core regions associated with the brain's default network (DMN), a network in which activity changes have been found in patients with anxiety disorders as compared with controls [Buckner et al., 2008; Maddock et al., 2003; Raichle et al., 2001; Zhao et al., 2007]. Alterations in PCC and MPFC activation have been linked to processing of emotionally salient stimuli, self-reflection, and self-processing activities [Cavanna and Trimble, 2006; Davey et al., 2016;

Raichle et al., 2001]. Hyperactivation in these regions might suggest that scene processing in the present study initiated mental imagery and autobiographical memory retrieval in PD patients [Buckner and Carroll, 2007; Spreng and Grady, 2009].

Emotion-related increased activation was revealed in the superior temporal gyrus region, which is part of a larger visual processing network and has been suggested to play an important role in social perception from visual cues [Allison et al., 2000]. This might suggest that PD patients compared with HC showed altered processing of social aspects such as intentions or implied movements for disorder-related versus neutral stimuli [Allison et al., 2000].

Dorsolateral PFC has generally been associated with top-down attentional control [Blasi et al., 2007; Comte et al., 2014] and suppression of emotion-expressive behavior [Phillips et al., 2008]. In line with that, PD patients revealed heightened right dorsolateral PFC activation when panic-related words were presented in an emotional Stroop [Van den Heuvel et al., 2005] or valence judgement task [Maddock et al., 2003].

The inferior frontal gyrus (IFG) is suggested to play a major role in regulatory processes which might indicate that PD patients recruited additional executive resources to avoid the expression of fear [Frank et al., 2014; Grecucci et al., 2013]. Note that Bystritsky et al. [2001] also observed enhanced IFG activation for PD in a disorder-related imagery paradigm. Thus, these additional findings in dorsolateral PFC and IFG complete the picture of frontal hyperactivation in the present PD sample.

No emotion-specific differences in functional connectivity between PD patients and HC could be unveiled by means of BSC, which speaks against our hypothesis of altered fronto-limbic connectivity in PD patients. Task-related connectivity research in PD is still in its infancy and our hypotheses were thus based on few studies, which all use conditioning paradigms. Differences in paradigms might explain differences in connectivity patterns, since fear responses elicited by aversive white noise differ from those obtained by disorder-related scenes [Kircher et al., 2013; Lueken et al., 2015]. Parameters that play an important role in connectivity analysis are the true functional connectivity difference, duration of inter-stimulus-interval, number of trial repetitions, duration of stimulus presentation, and variability of hemodynamic response function [Cisler et al., 2014]. Thus, further studies on task-related functional connectivity in PD are needed to associate findings from regions of interest with findings of connectivity analyses.

Limitations

Although the present sample of unmedicated PD patients can be considered large in the field of clinical affective neuroscience, even greater samples are needed to

boost statistical power and improve reliability of findings, especially with respect to connectivity analysis. Differences across patients in number and severity of comorbid conditions present a limitation for the present study. However, due to the high prevalence of comorbidities in PD (found in ~80% of patients [Kessler et al., 2006]), the inclusion of patients with comorbid conditions per se increases external validity. It has to be kept in mind that the present study only addresses visual disorder-related scene processing. It remains to be elucidated whether these results conform with data from disorder-related processing in other modalities, and in how far a model of disorder-related processing can be developed independently from stimulus modality.

CONCLUSIONS

The present novel, disorder-related, and tailor-made stimulus set is ideally suited to investigate treatment effects in PD as it is characterized by the relevance and naturalness of the stimuli, which increases the sets ecological validity [Schmuckler, 2001]. With our present stimulus set, future studies might unveil whether altered disorder-related processing serves as a predictor of treatment response. The novel set of disorder-related complex visual scenes elicited anxiety in PD, as reflected in differential ratings. Processing of these highly ecologically valid scenes was associated with brain activations in the extended fear circuitry of PD. Higher brainstem activation even went along with higher levels of subjective anxiety, underlining that disorder-related scene processing triggers basal mechanisms of fear, and leads to subjective levels of anxiety. It is the first affective processing study in PD that shows a direct link between subjective anxiety evoked by external stimuli and altered activation in the homeostatic alarm system. Based on the present fMRI results, we suggest increased emotion-specific processing and interoceptive awareness to play a major role in disorder-related visual processing in PD.

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STIMULUS SET PAPS-M

The stimulus set is available for use by the scientific community. For further information please contact katharina.feldker@uni-muenster.de.

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